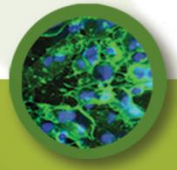
Cellular Dynamics international

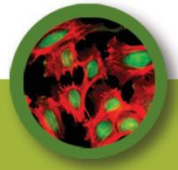
Use of Stem Cell-derived Cardiomyocytes and Real Time Impedance – based Measurements to Predict Drug-Induced QT prolongation and Arrhythmia

Kyle Kolaja

March 7th, 2014

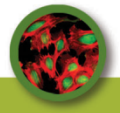






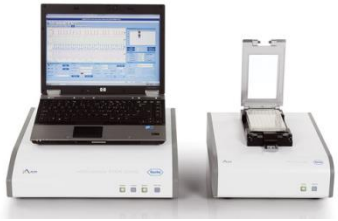
Outline of Talk

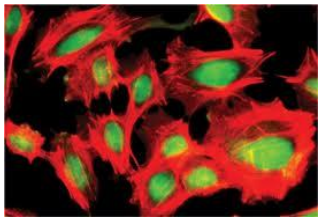
- **Background on the Project**
- **Device**
- **Cells**
- **Assay**
- **Contract Providers**
- **Conclusions**



Project Summary

- **Overview of Technology**
  - A three party-development project
    - Roche Applied Sciences who at the time had licensed ACEA xCelligence impedance products
    - Cellular Dynamics International
    - Roche Pharma





- **Robust, reproducible in vitro assay for arrhythmia**
  - Widely cited paper (**48x** as 2/28/14)
  - Adopted by numerous pharma
  - Offered at several CROs
  - Part of a larger effort to change TQT (E14) and in vitro safety pharmacology guidance (ICH7B)

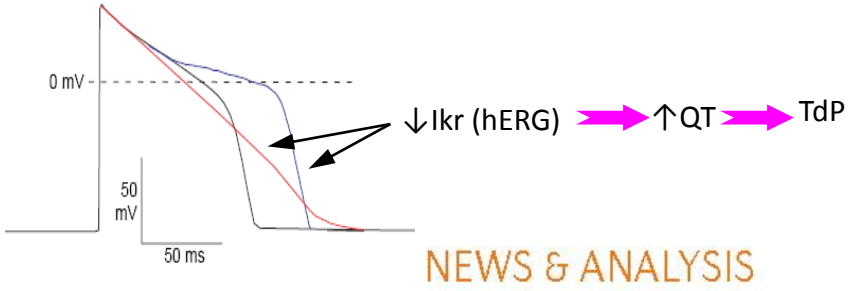
TOXICOLOGICAL SCIENCES 123(1), 281–289 (2011)
doi:10.1093/toxsci/kfr158
Advance Access publication June 20, 2011

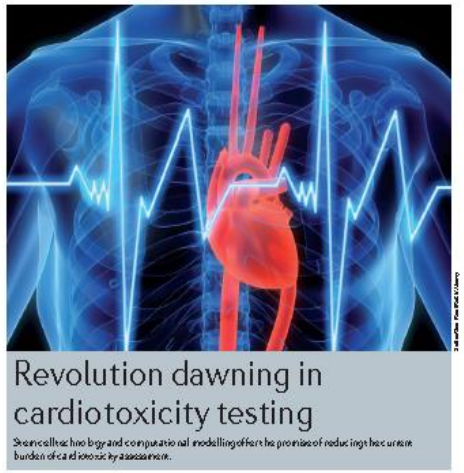
Estimating the Risk of Drug-Induced Proarrhythmia Using Human Induced Pluripotent Stem Cell–Derived Cardiomyocytes

Liang Guo, Rory M. C. Abrams, Joshua E. Babiarz, Jennifer D. Cohen, Sei Kameoka, Martin J. Sanders, Eric Chiao, and Kyle L. Kolaja[1]

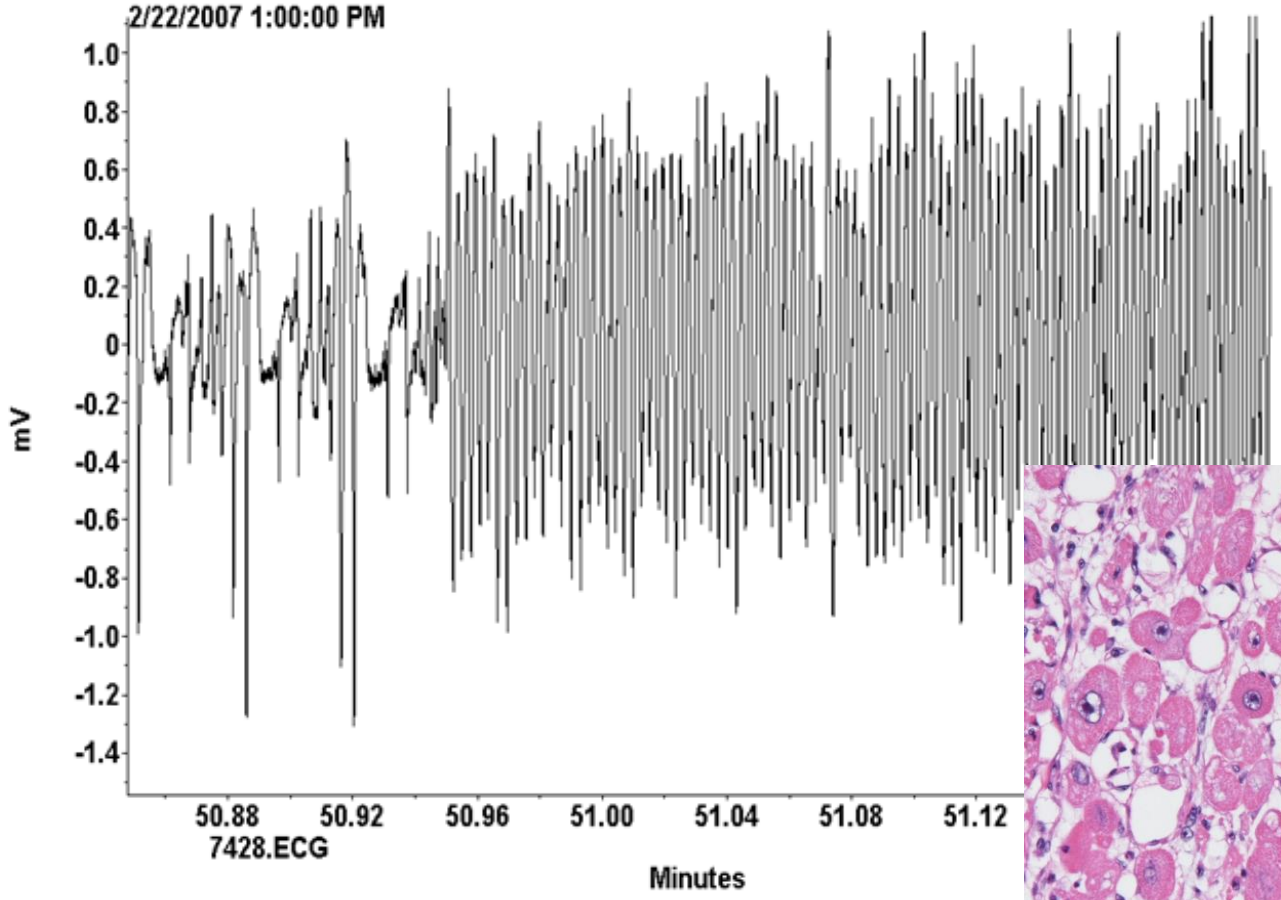
Cardiovascular Safety Pharmacology

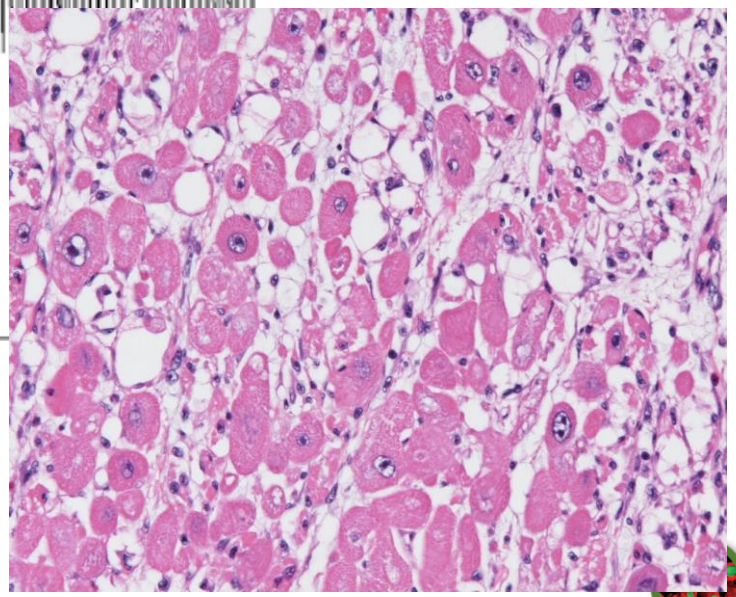
- **~10 drugs pulled from market based on torsades de pointe**
- **Regulatory Guidance documents ICH7A and 7B proscribe a new host of cardiovascular safety approaches**
  - hERG screening – ex vivo preps – in vivo animal models – ECGs – Thorough QTc trials
- **Two consequences –**
  - no drug induced torsades
  - a lot of beneficial drugs not marketed
- **Early screening relies heavily on hERG**
  - hERG block ≠ QT prolongation
  - hERG block ≠ arrhythmia
  - QT prolongation ≠ arrhythmia
  - Arrhythmia can be independent of hERG
- **Cardiovascular safety and regulations could be better**
  - HESI and CIPA





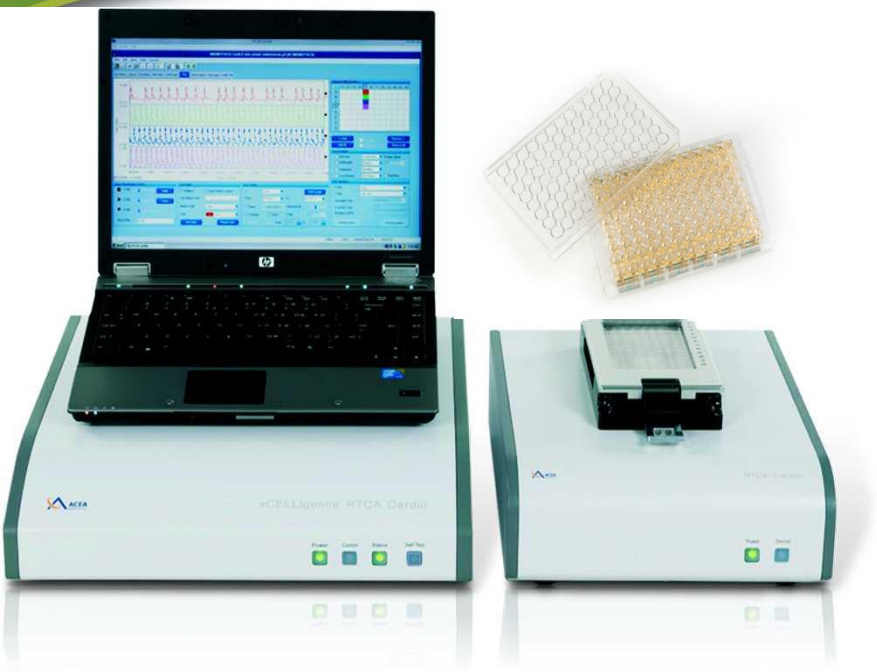
The Genesis of the Project

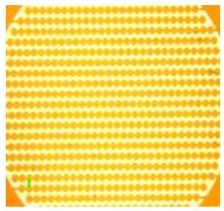


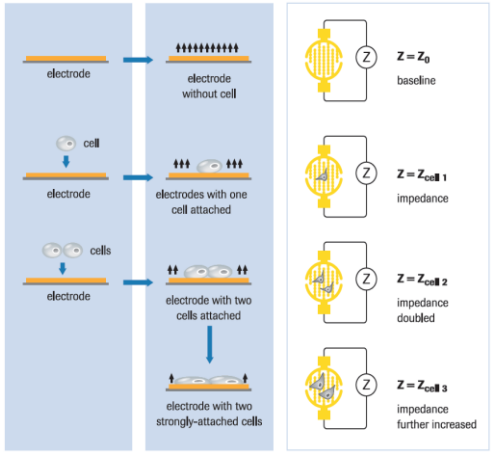


Cellular Dynamics international

xCELLigence RTCA-Cardio System



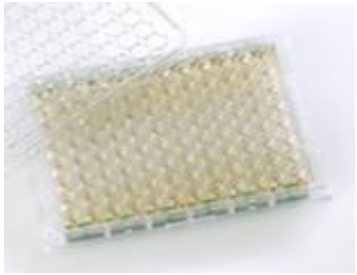




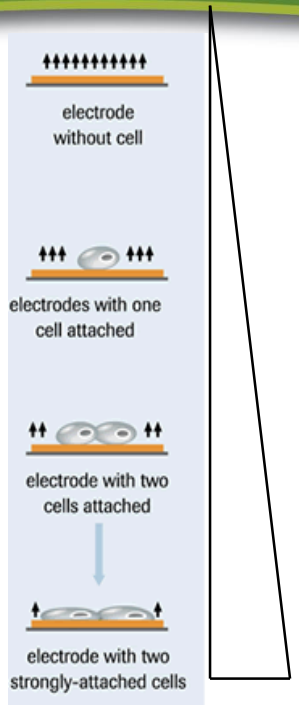
- **Real time, non-destructive, continuous data**
- **Monitors cardiotoxicity and cardiomyocyte beating**
- **Highly sensitive**
- **Simultaneously assesses**
  - cell viability
  - properties related to contraction
- **96 well format**
- **Enables short term (msec) and long term (days or weeks) measurements in a single assay**

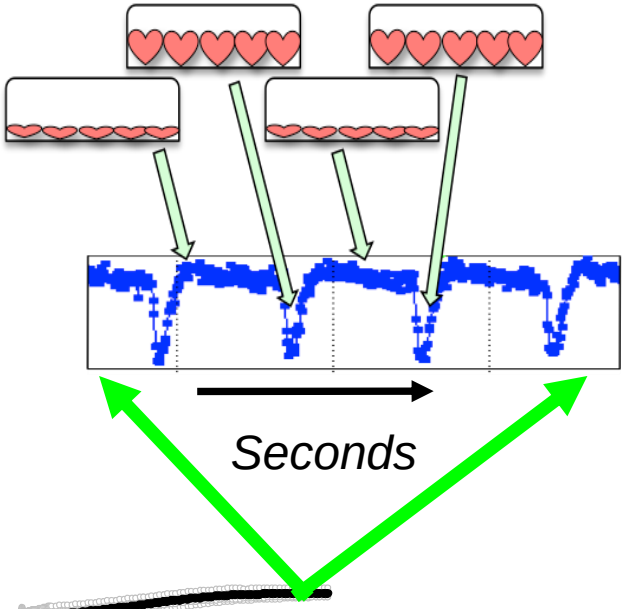
- **Why Impedance vs MEA?**
  - Collaboration with Roche Applied Sciences
  - At the time, higher throughput (96 vs 6)
  - Treat and collect data for days vs hours

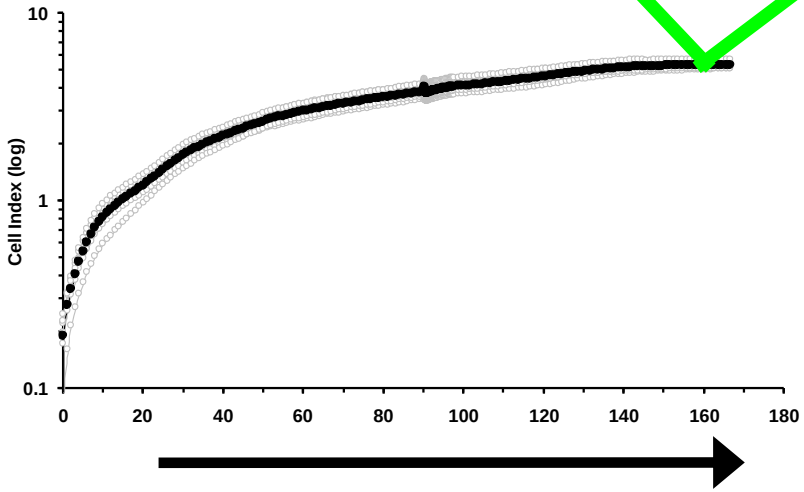
Impedance Detection of Cardiomyocyte Beating



96-well E-eplate



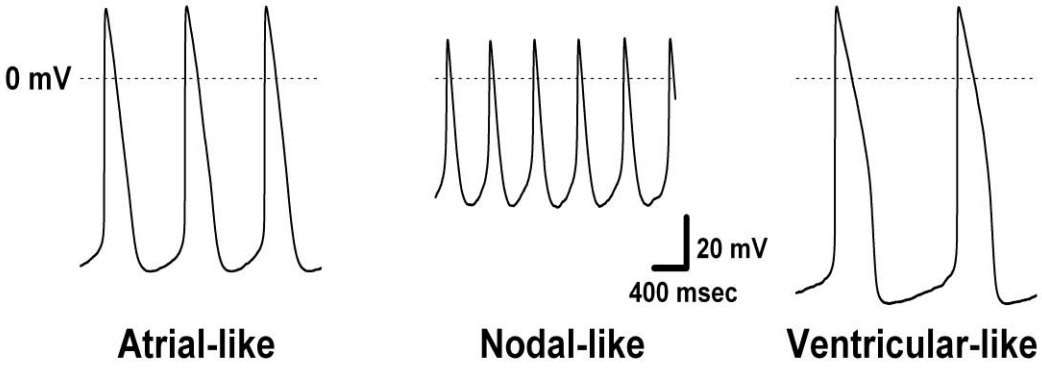




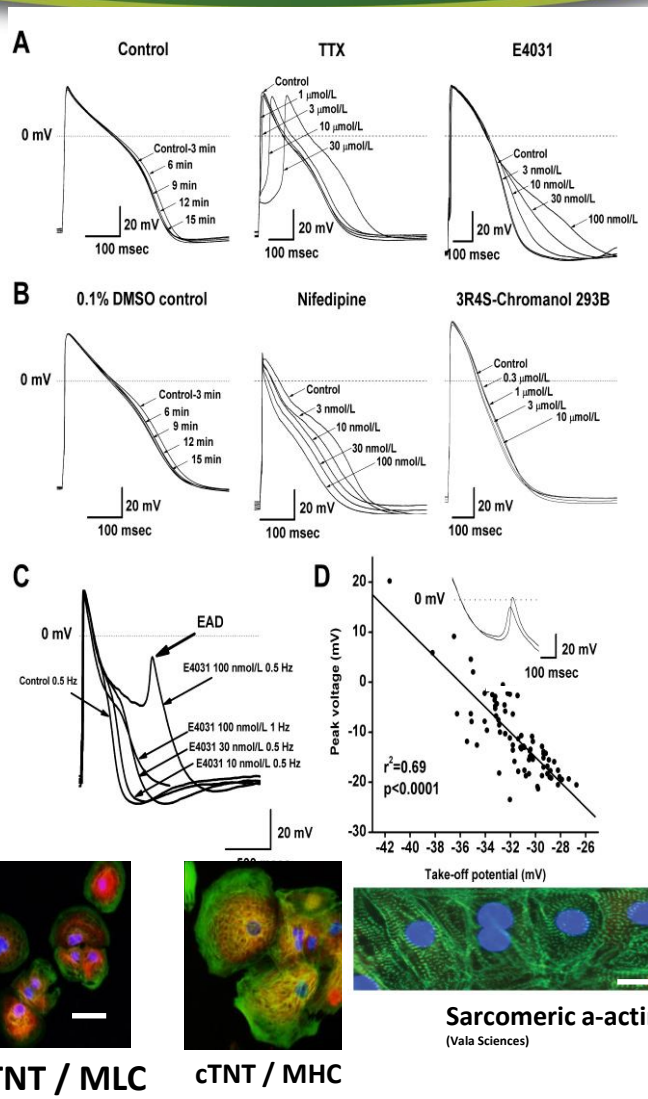
Confirmation of Cardiomyocyte Nature

hIPs Cardiomyocytes

- Express major cardiac proteins
- Exhibit cardiac action potential wave forms
- Functionally active cardiac channels
- Respond as expected to channel blockers



APs (n=59)	Interval (sec)	MDP (mV)	Peak (mV)	Amp (mV)	dV/dt_{max} (V/sec)	APD10 (msec)	APD30 (msec)	APD90 (msec)	APD30-40 /APD70-80
Atrial-like (n=13)	1.2±0.2	-73.5±1.5	26.7±1.4	100.2±2.1	26.2±3.9	60.8±5.5	123.1±10.3	286.2±21.2	1.1±0.1
Nodal-like (n=14)	1.0±0.1	-60.1±2.4	19.5±2.0	79.6±2.9	5.5±0.4	65.3±18.4	111.8±15.9	254.7±20.5	1.0±0.1
Ventricular-like (n=32)	1.7±0.1	-75.6±1.2	28.3±1.0	104.0±1.1	27.8±4.8	74.1±4.8	180.0±10.7	414.7±21.8	2.5±0.2

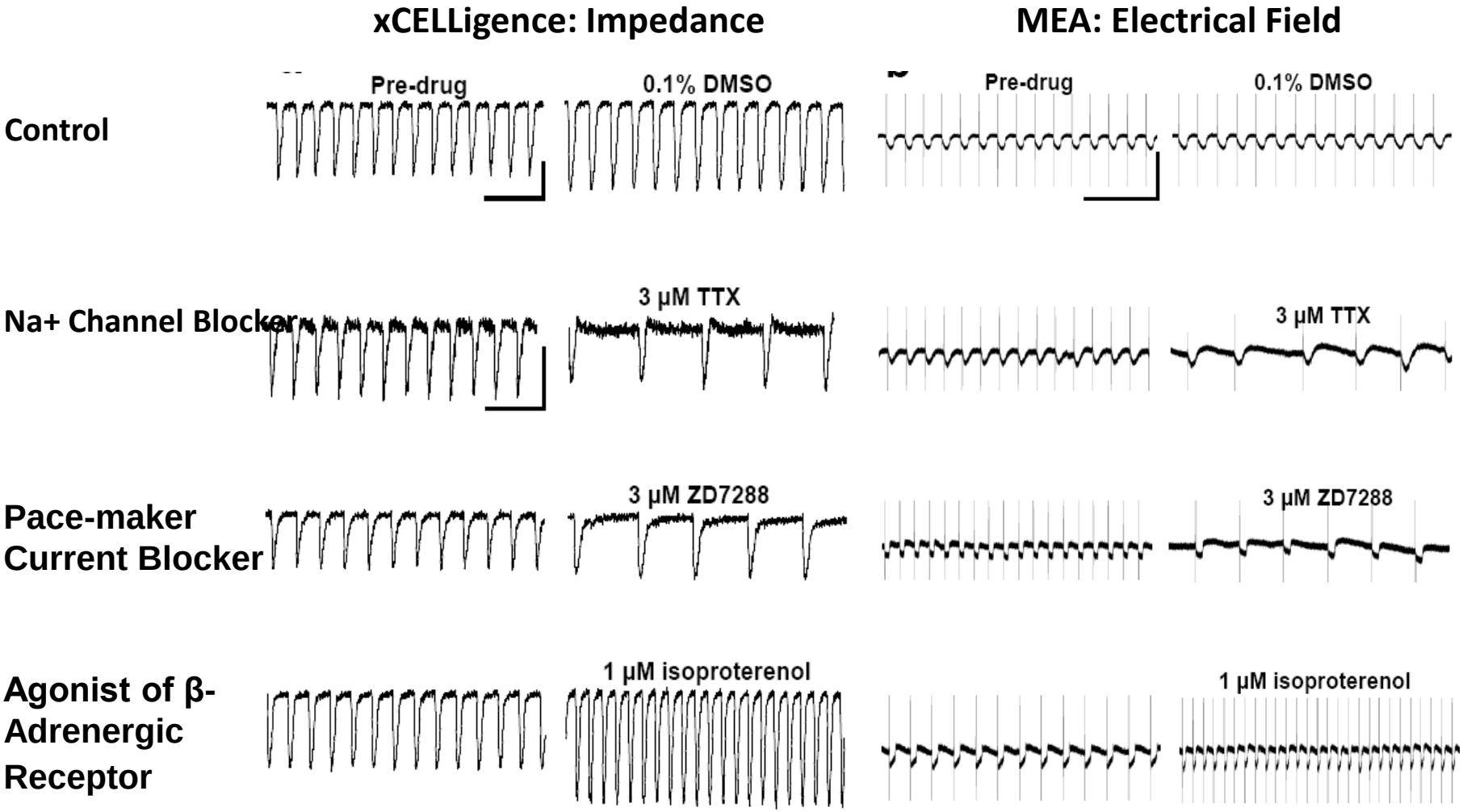


Ma et. al Am J Physiol Heart Circ Physiol. (2011) High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents

Cellular Dynamics international

Drug-induced changes in beat rate

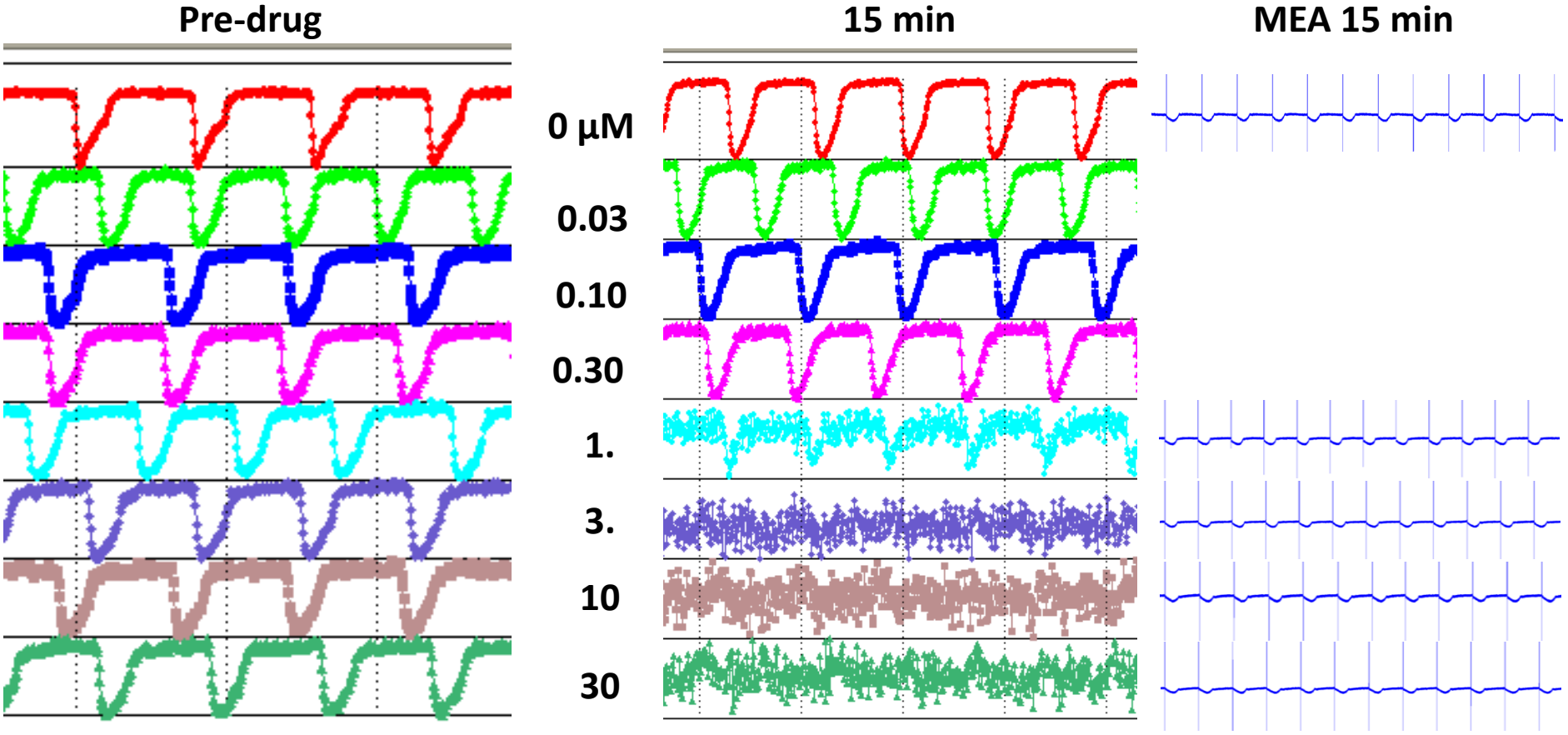
Indication of functional cardiac ion channels



Does impedance detect the physical beating or the electrical changes?

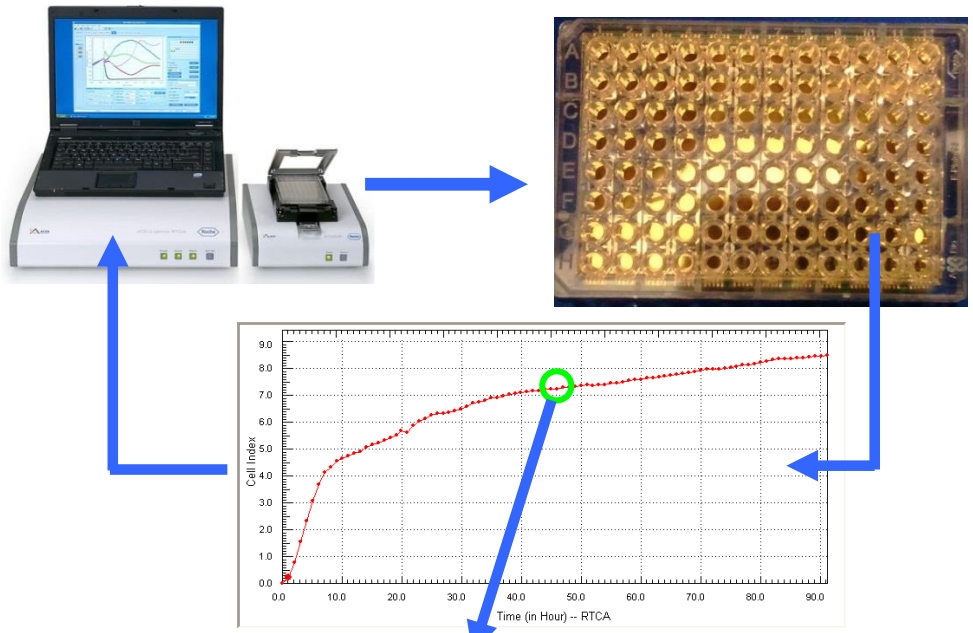
Blebbistatin:

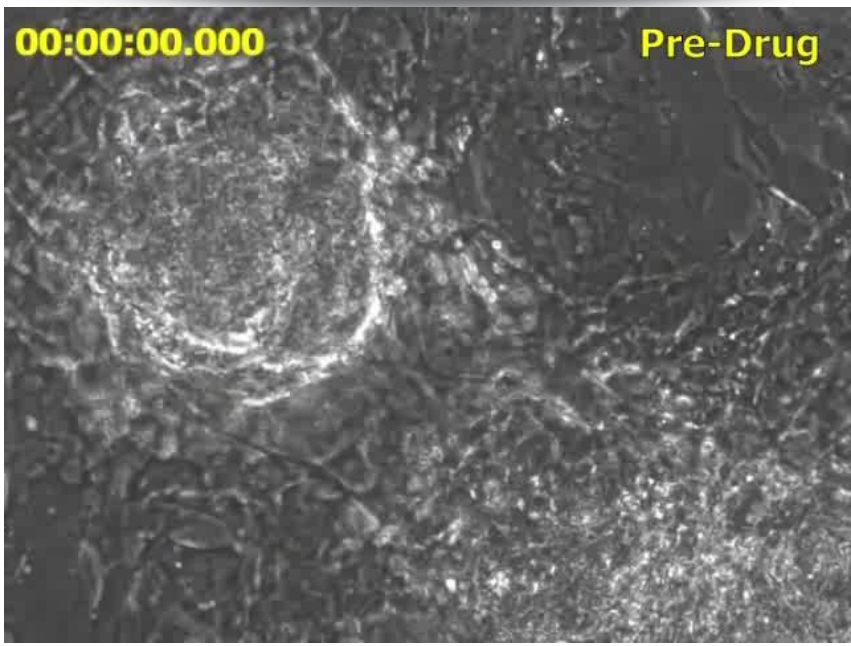
Blocks myosin II in an actin-detached state



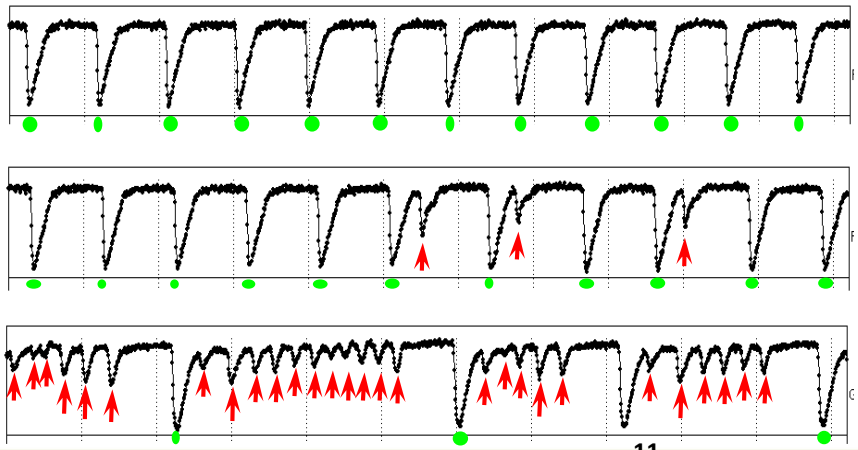
Human Cardiomyocytes Arrhythmia Risk (hCAR) Model

iPSC derived human cardiomyocytes





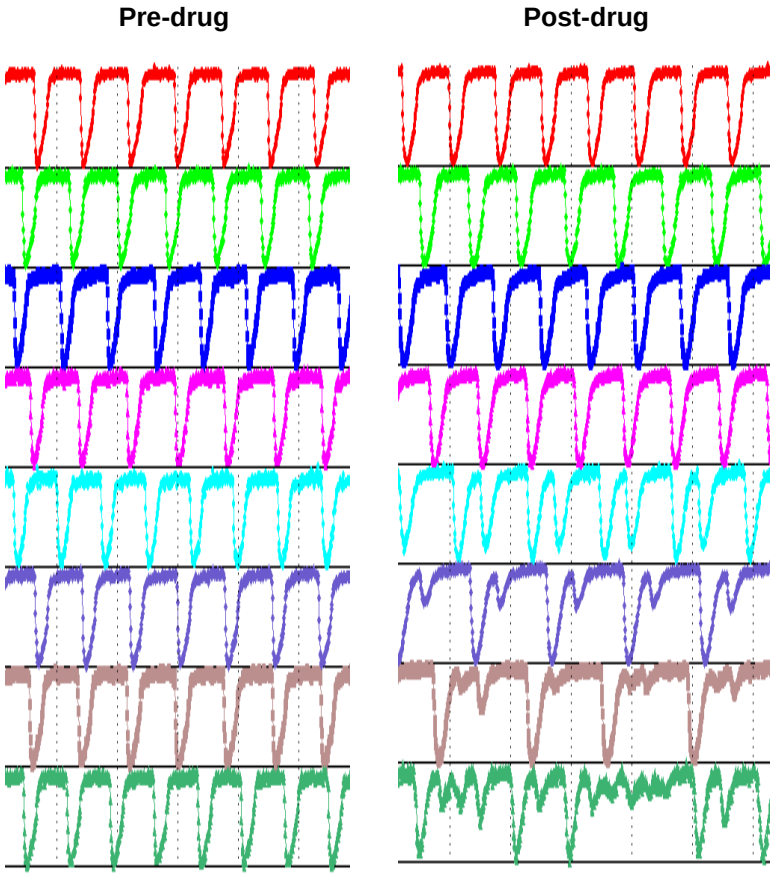
R	I	T	IBR
12	0	12	0
11	3	14	0.21
4	28	32	0.88

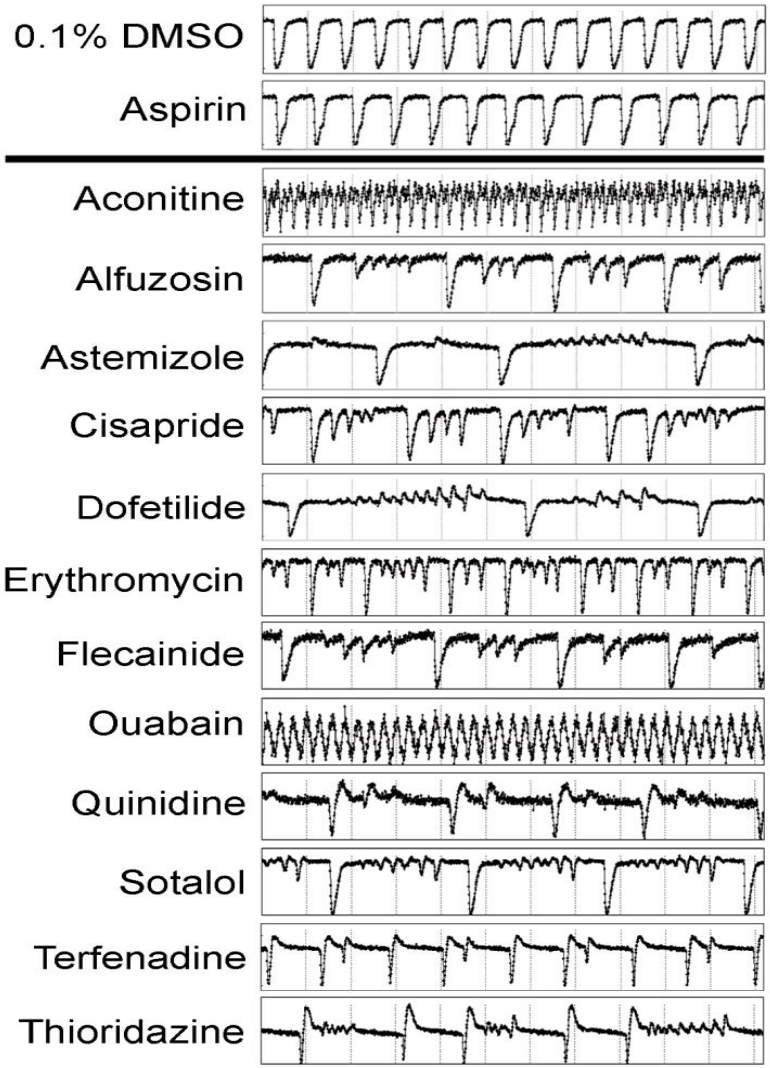


IB20= lowest tested concentration resulting in 20% irregular beats

Cellular Dynamics international

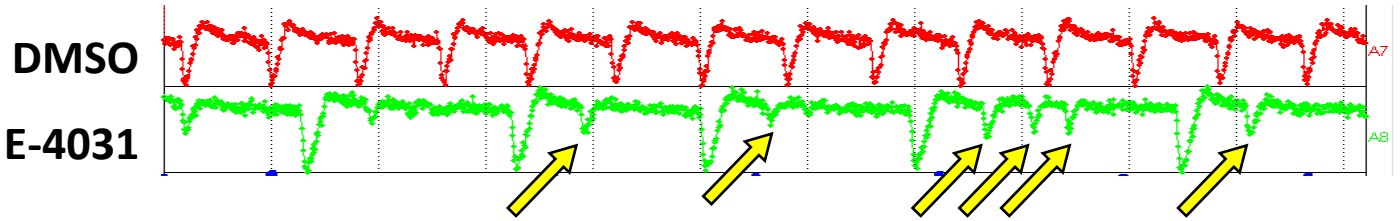
In vitro Detection of Arrhythmias with Human iPSC-Derived Cardiomyocytes



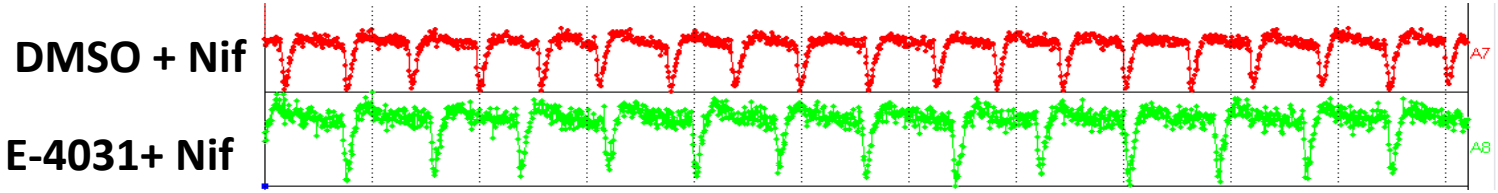


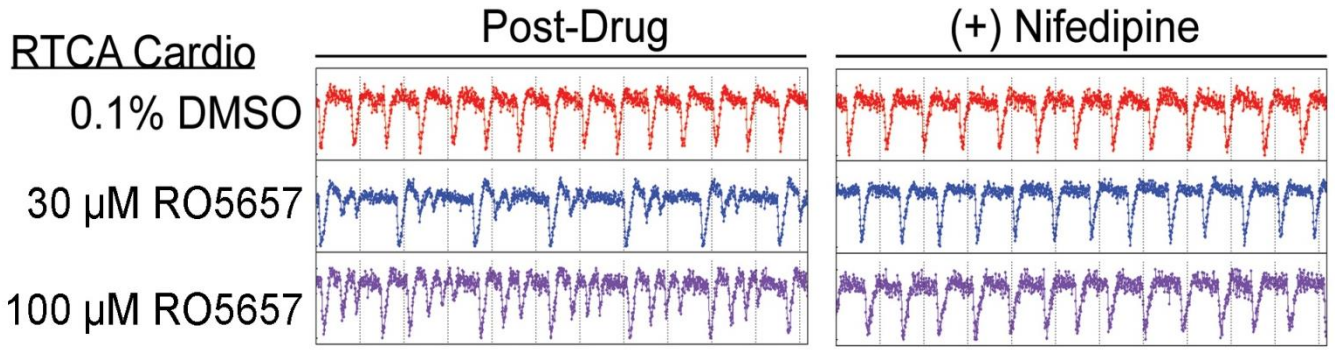
Impedance Arrhythmia Rescue with Calcium Channel Block

E-4031 causes arrhythmia



Nifedipine rescues E-4031-induced arrhythmia





Functional Rescue of Drug-induced Arrhythmia

Cellular Dynamics international

hCAR Initial Assay Validation: determine in vitro to *in vivo correlation*

- 12 Pro-arrhythmic
- 11 Non-arrhythmic
- **IB20 30 uM**
  - **One False Positive**
  - **No False Negatives**

- **IB20= lowest tested concentration resulting in 20% irregular beats**

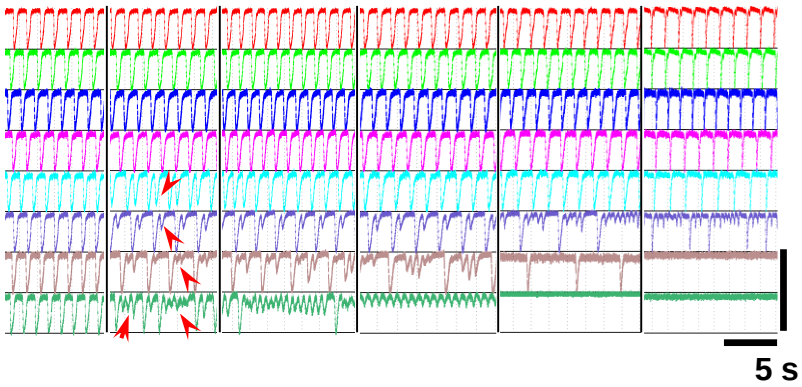
Drug	IB20 (µM)	hERG	QT	Clinical arrhythmia
Dofetilide	0.003	(+)	(+)	(+)
Ouabain	0.03	(-)	(-)	(+)
Aconitine	0.03	(-)	(-)	(+)
Cisapride	0.03	(+)	(+)	(+)
E-4031	0.03	(+)	(+)	(+)
Astemizole	0.03	(+)	(+)	(+)
Terfenadine	0.3	(+)	(+)	(+)
Flecainide	1	(+)	(+)	(+)
Alfuzosin	1	(-)	(+)	(-)
Thioridazine	3	(+)	(+)	(+)
Quinidine	10	(+)	(+)	(+)
Erythromycin	30	(+)	(+)	(+)
Sotalol	30	(+)	(+)	(+)
Fluoxetine	>30	(+)	(+)	(-)
Verapamil	>30	(+)	(±)	(-)
Moxifloxacin	>100	(+)	(+)	(+)
Amiodarone	>100	(+)	(+)	(±)
Ranolazine	>100	(+)	(+)	(-)
Captopril	>100	(-)	(-)	(-)
Rofecoxib	>100	(-)	(-)	(-)
Amoxicillin	>1000	(-)	(-)	(-)
Aspirin	>1000	(-)	(-)	(-)
Nifedipine	>3	(-)	(-)	(-)

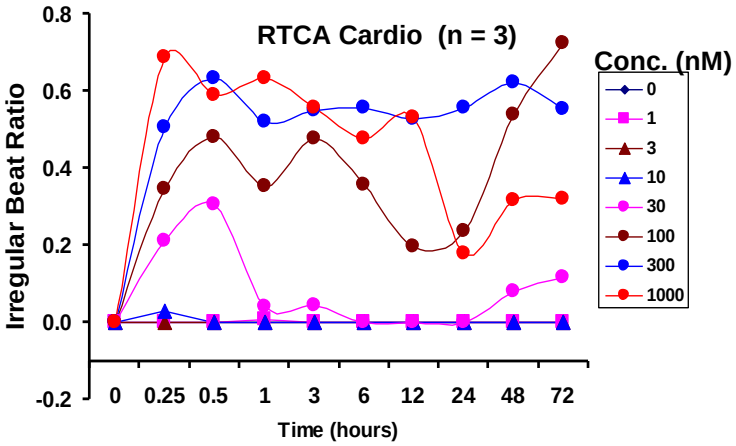
Guo et al. Toxicol Sci. 2011 Sep;123(1):281-9.

Cellular Dynamics international

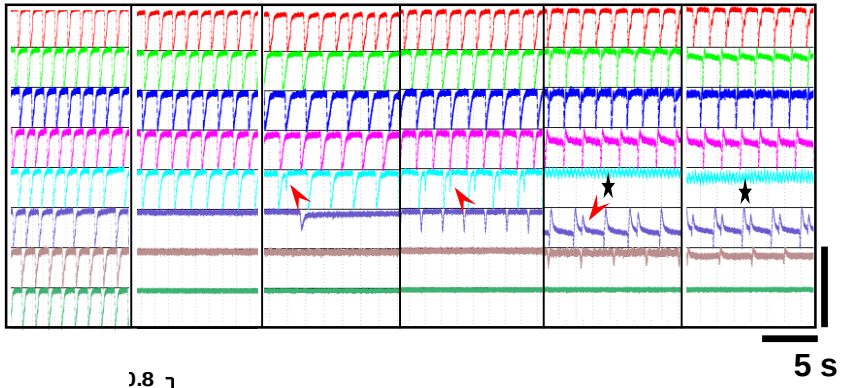
hCAR Assay Validation: Onset time of IB20 varies

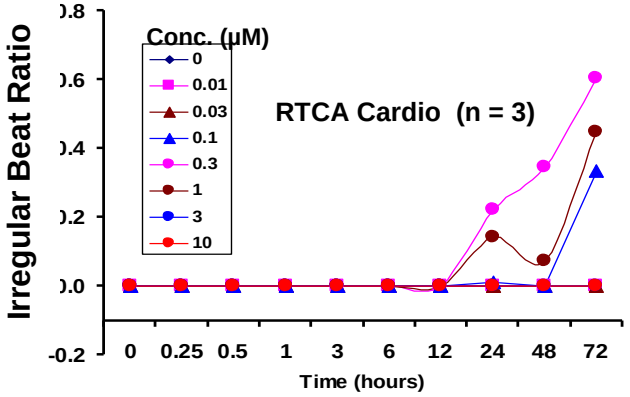
E-4031





Terfenadine

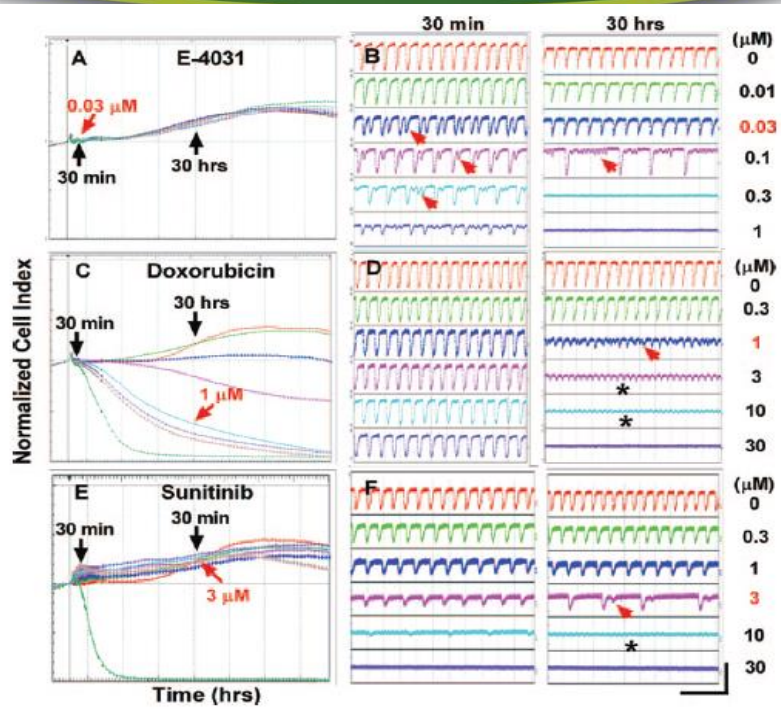




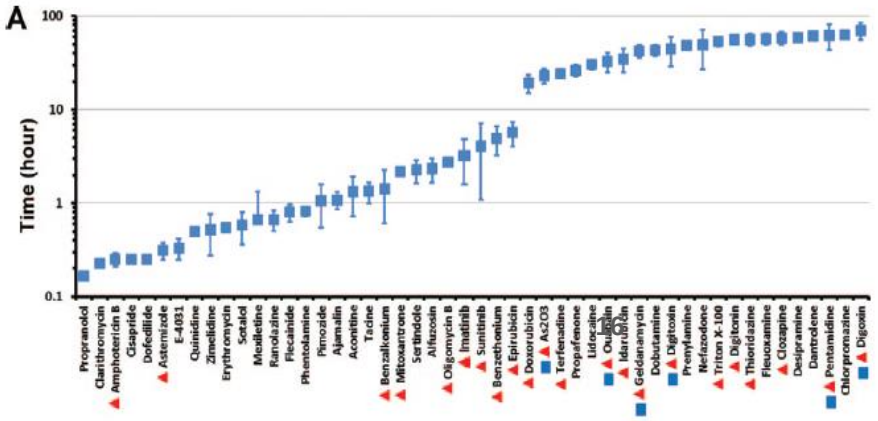
- Rapid sustained effect of E-4031 on arrhythmia
- Terfenadine - with arrest of beating and late onset arrhythmia
- Longer term assessment improves accuracy

hCAR Second Paper Goals

- **Expand the dataset**
  - More positives and negatives, toxicants, hERG trafficking inhibitors
- **Fine tune the prediction**
  - delayed beat rate in vitro predicting QT prolongation
  - atypical beats in vitro predicting arrhythmia
  - Examine onset time of IB20
- **Improve the metrics**
  - IB20 as well as threshold cutoffs
  - In vitro efficacy benchmarks
- **Investigate potential confounding effects of cytotoxicity**



A MODEL FOR CARDIAC ARRHYTHMIA PREDICTION



TOXICOLOGICAL SCIENCES
doi:10.1093/toxsci/kft205
Advance Access publication September 18, 2013

Refining the Human iPSC-Cardiomyocyte Arrhythmic Risk Assessment Model

Liang Guo,[1] Luke Coyle, Rory M. C. Abrams,[2] Raymond Kemper,[3] Eric T. Chiao,[4] and Kyle L. Kolaja[5,6]

Cellular Dynamics international

Human Cardiomyocytes Arrhythmia Risk (hCAR) Model

Next Round of Validation

Drug	C_{max} (nM)	IB_{50} (μM)	↓hERG	↑QT	TdP
Digitoxin	33	0.003	(-)[f]	(-)	(+)[r]
Dofetilide	6	0.003	(+)	(+)	(+)
Digoxin	3	0.01	(-)[f]	(-)	(+)[r]
Ouabain	170	0.01	(-)[f]	(-)	(+)[r]
Aconitine	77	0.03	(-)	(-)	(+)[r]
Astemizole	8	0.03	(+)	(+)	(+)
E-4031	13	0.03	(+)	(+)	(+)
Pimozide	217	0.1	(+)	(+)	(+)
Sertindole	3.18	0.1	(+)	(+)	(+)
Cisapride	129	0.3	(+)	(+)	(+)
Geldanamycin	16,800	0.3	(-)[f]	(-)	(+)[r]
Idarubicin	123	0.3	(-)	(+)	(+)[r]
Terfenadine	300	0.3	(+)	(+)	(+)
Alfuzosin	56	1	(-)	(+)	(-)
Dobutamine	3,819	1	(+)	(-)	(-)
Doxorubicin	15,344	1	(-)	(+)	(+)[r]
Flecainide	1,931	1	(+)	(+)	(+)
Pentamidine	2,181	1	(-)[f]	(+)	(+)
Tacrine	100	1	(-)	(-)	(-)
Amphotericin B	89.818	3	(-)	(+)	(+)[r]
Arsenic Trioxide	12,132	3	(-)[f]	(+)	(+)
Clozapine	1	3	(-)	(+)	(±)
Mitoxantrone	3,311	3	(-)	(+)	(+)[r]
Prenylamine	70	3	(-)	(+)	(+)
Sunitinib	253	3	(+)	(+)	(+)
Thioridazine	1,781	3	(+)	(+)	(+)
Zimelidine	328	3	(+)	(±)	(±)
Ajmaline (i.v.)	105	10	(+)	(+)	(+)
Chlorpromazine	2,630	10	(+)	(+)	(+)
Clarithromycin	6,025	10	(+)	(+)	(+)
Dantrolene	7,900	10	(-)	(-)	(-)
Desipramine	601	10	(+)	(+)	(+)
Epirubicin	16,036	10	(-)	(+)	(+)[r]
Nefazodone	4,858	10	(+)	(+)	(-)
Phentolamine	100	10	(-)	(-)	(-)
Quinidine	21,578	10	(+)	(+)	(+)
Erythromycin (i.v.)	34,064	30	(+)	(+)	(+)
Fluvoxamine	1,257	30	(+)	(-)	(-)
Imatinib	3,541	30	(-)	(+)	(-)
Mesoridine	11,161	30	(+)	(-)	(-)
Propafenone	4,827	30	(+)	(+)	(+)
Propranolol (i.v.)	193	30	(-)	(+)	(-)
Sotalol	14,733	30	(+)	(+)	(+)

	in vivo ECG			hERG		
Cyclosporin A	1,498	100	(-)	(-)	(-)	
Lidocaine (i.v.)	36,000	100	(+)	(-)	(-)	
Pimobendan	164	100	(-)	(-)	(-)	
Ranolazine	6,009	100	(+)	(+)	(-)	
Nifedipine	194	>3	(-)	(-)	(-)	
Amitriptyline	793	>30	(-)	(+)	(±)	
Bepridil	3,298	>30	(+)	(+)	(+)	
Cetirizine	800	>30	(-)	(-)	(-)	
Cibenzoline	2,168	>30	(+)	(+)	(-)	
Delta pridine	493	>30	(-)	(-)	(-)	
Diltiazem	552	>30	(-)	(-)	(-)	
Diphenhydramine	157	>30	(-)	(+)	(+)	
Fluorouracil	4,613	>30	(-)	(-)	(+)[r]	
Fluoxetine	485	>30	(+)	(+)	(+)	
Imipramine	1,070	>30	(+)	(+)	(±)	
Ketoconazole	17,689	>30	(+)	(+)	(±)	
Loratadine	23	>30	(-)	(-)	(-)	
Nitrendipine	150	>30	(-)	(-)	(-)	
Olanzipine	74	>30	(-)	(±)	(-)	
Rosiglitazone	1,673	>30	(-)	(-)	(-)	
Troglitazone	6,387	>30	(-)	(-)	(-)	
Verapamil	815	>30	(+)	(-)	(-)	
Acetamidophenol	130,000	>100	(-)	(-)	(-)	
Alpidem	284	>100	(-)	(-)	(-)	
Amiodarone	3,874	>100	(+)	(+)	(±)	
Atenolol	1,284	>100	(-)	(-)	(-)	
Captopril	2,466	>100	(-)	(-)	(-)	
Colchicine	16	>100	(-)	(-)	(-)	
Cyclophosphamide	153,200	>100	(-)	(±)	(-)	
Dexamethasone	136,052	>100	(-)	(-)	(-)	
Levamisole	136	>100	(-)	(-)	(-)	
Mechlorethamine	?	>100	(-)	(-)	(-)	
Moxifloxacin	10,276	>100	(+)	(+)	(+)	
Nimesulide	15,000	>100	(-)	(-)	(-)	
Pemoline	6,525	>100	(-)	(-)	(-)	
Rofecoxib	1,021	>100	(-)	(-)	(-)	
Tolcapone	21,959	>100	(-)	(-)	(-)	
Zalcitabine	115	>100	(-)	(-)	(-)	
Amoxicillin	17,036	>1000	(-)	(-)	(-)	
Aspirin	10,000	>1000	(-)	(-)	(-)	

30	-	-	-	4.9		
00	-	-	-	30		
27	>100	>100	-	-	-	4.0
28	>100	>100	-	-	-	4.3
29	>100	>100	-	-	-	30
30	>100	>100	-	-	-	>300

83 Compounds

~82% -- arrhy. prediction
>90% -- QT prediction

30 Internal Compounds

80% -- arrhy. prediction
95% -- QT prediction

Internal and External Validation

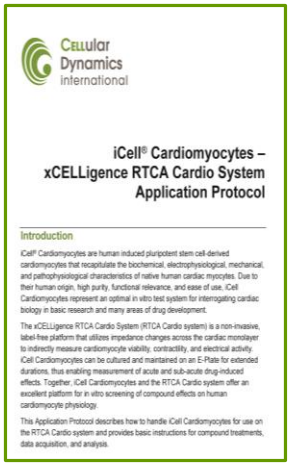
- **Assay**
- Confirmation of effects using MEA
- Confirmation of dose response
- Multiple time points

- **Cells**
- Express relevant ion channels and are functionally similar
- Respond to known drug standards
- Assessment of lot to lot performance
- Assessment of interassay drug response

- **Publications**
  - Cross-site, cross-investigator correlation

- **Industry Adoption**
- CDI developed standard protocol
  - Extensive lot to lot characterization
- Pharma and CROs have run internal assessments

- **Unknown/Test Compound**
- Inclusion of multiple dose and time points
- Inclusion of at least 1 positive control


Cellular Dynamics international

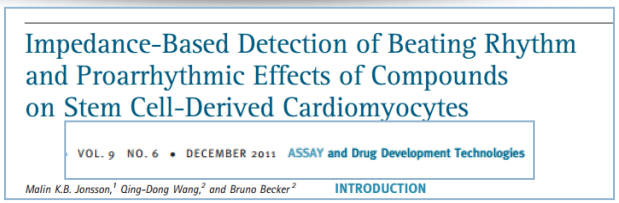
iCell® Cardiomyocytes – xCELLigence RTCA Cardio System Application Protocol

Introduction

iCell® Cardiomyocytes are human induced pluripotent stem cell-derived cardiomyocytes that recapitulate the biochemical, electrophysiological, mechanical, and pathophysiological characteristics of native human cardiac myocytes. Due to their human origin, high purity, functional relevance, and ease of use, iCell Cardiomyocytes represent an optimal in vitro test system for interrogating cardiac biology in basic research and many areas of drug development.

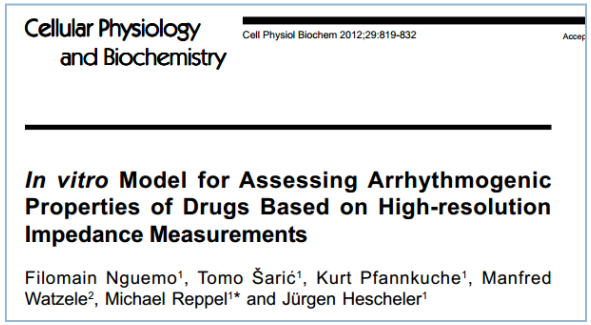
The xCELLigence RTCA Cardio System (RTCA Cardio system) is a non-invasive, label-free platform that utilizes impedance changes across the cardiac monolayer to indirectly measure cardiomyocyte viability, contractility, and electrical activity. iCell Cardiomyocytes can be cultured and maintained on an E-Plate for extended durations, thus enabling measurement of acute and sub-acute drug-induced effects. Together, iCell Cardiomyocytes and the RTCA Cardio system offer an excellent platform for in vitro screening of compound effects on human cardiomyocyte physiology.

This Application Protocol describes how to handle iCell Cardiomyocytes for use on the RTCA Cardio system and provides basic instructions for compound treatments, data acquisition, and analysis.


Impedance-Based Detection of Beating Rhythm and Proarrhythmic Effects of Compounds on Stem Cell-Derived Cardiomyocytes

VOL. 9 NO. 6 • DECEMBER 2011 ASSAY and Drug Development Technologies

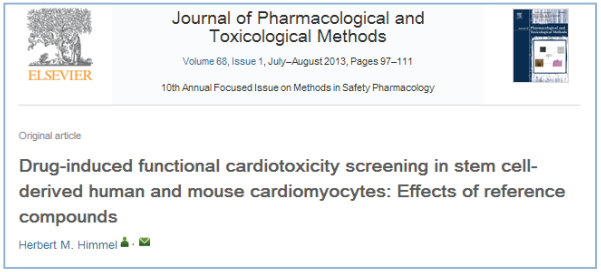
Malin K.B. Jonsson,[1] Qing-Dong Wang,[2] and Bruno Becker[2] INTRODUCTION


Cellular Physiology and Biochemistry

Cell Physiol Biochem 2012;29:819-832

***In vitro* Model for Assessing Arrhythmogenic Properties of Drugs Based on High-resolution Impedance Measurements**

Filomain Nguemo[1], Tomo Šarić[1], Kurt Pfannkuche[1], Manfred Watzele[2], Michael Reppel[1]* and Jürgen Hescheler[1]


ELSEVIER

Journal of Pharmacological and Toxicological Methods

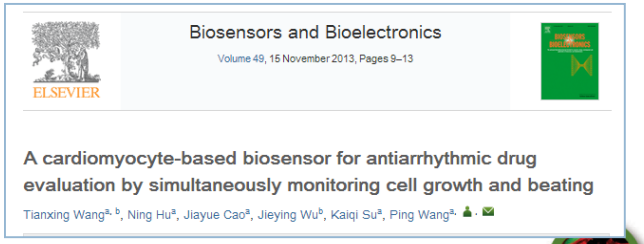
Volume 68, Issue 1, July–August 2013, Pages 97–111

10th Annual Focused Issue on Methods in Safety Pharmacology

Original article

Drug-induced functional cardiotoxicity screening in stem cell-derived human and mouse cardiomyocytes: Effects of reference compounds

Herbert M. Himmel

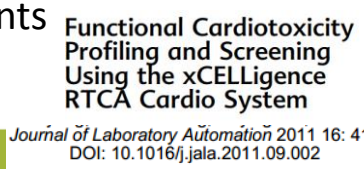

ELSEVIER

Biosensors and Bioelectronics

Volume 49, 15 November 2013, Pages 9–13

A cardiomyocyte-based biosensor for antiarrhythmic drug evaluation by simultaneously monitoring cell growth and beating

Tianxing Wang[a, b], Ning Hu[a], Jiayue Cao[a], Jieying Wu[b], Kaiqi Su[a], Ping Wang[a]


Functional Cardiotoxicity Profiling and Screening Using the xCELLigence RTCA Cardio System

Journal of Laboratory Automation 2011 16: 415
DOI: 10.1016/j.jala.2011.09.002

Human Cardiomyocyte Arrhythmia Risk (hCAR) Model: Summary

- **Highly predictive in vitro model to identifies compounds that cause QT prolongation and/or arrhythmia clinically**
  - High through-put, real-time, non-destructive
  - Minimal compound requirements, fast turn-around, low technical rigor
    - Longer experiments identify indirect arrhythmia mechanisms
- **Provides context to hERG inhibition values**
- **Verification/Validation of technology and cell performance**
  - Across sites
  - Across labs
  - Across compounds
  - Across lots of cells